



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

**BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES**

Appeal No. _____

BRIEF FOR APPLICANT (APPELLANT)

Ex parte Hitoshi Matsumoto *et al.*

**COMPOSITIONS FOR FOODS, PROCESS FOR PRODUCING THE SAME AND
FUNCTIONAL FOODS AND DRINKS CONTAINING THE SAME**

Serial No. 10/019,402, filed December 28, 2001

Examiner: Donna Jagoe

Group Art Unit: 1614

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INTRODUCTION

This is an appeal from the final rejection of claims 27-30, 32-40, 44 and 49. The Final Office Action mailed March 11, 2008, set forth a single prior art rejection of Appellant's claims under 35 U.S.C 103(a) as being unpatentable over Nakhmedov *et al.* (1975) (Konservnaya I Ovoshchesushil'naya Promyshlennost; hereinafter referred to as "Nakhmedov") in view of U.S. Patent 4,643,902 of Lawhon *et al.* ("Lawhon") and Laboratories Chibret, British Patent Specification no. 1,007,751 ("British '751").

A Notice of Appeal was filed in this application on August 11, 2008. An Advisory Action was mailed on October 16, 2008, which indicated that the Examiner did not enter the amendments filed after final rejection and maintained the prior art rejections. This Appeal Brief is being filed with a Two-Month Extension of Time, namely, on Tuesday, December 9, 2008.

I. REAL PARTY IN INTEREST

The real party in interest is the owner of this application. At the time of the filing of this brief, the present application was owned by the assignee, Meiji Seika Kaisha, Ltd.

II. RELATED APPEALS AND INTERFERENCES

There are no related appeals or interferences that either directly affect or have a bearing on the decision in this appeal.

III. STATUS OF CLAIMS

All pending claims 27-30, 32-40, 44 and 49 are on appeal and are listed in the Claims Appendix at the end of this brief. Claims 1-26, 31, 41-43 and 45-48 were canceled during the prosecution of the application and are therefore not listed in the Claims Appendix. There are no other claims present in the application.

IV. STATUS OF AMENDMENTS

Appellant filed a Response after Final on August 11, 2008 making editorial changes to the claims that simplified the claims for appeal; namely, changing independent claim 29 into a dependent claim and dependent claim 30 into an independent claim. In the Advisory Action mailed October 16, 2008, the Examiner refused entry of these amendments, stating that the proposed amendments raise new issues that would require further consideration and/or search. Accordingly, claims 27-30, 32-40, 44 and 49 as listed in the Claim Appendix correspond to the claims presented in this application at the time of the final rejection.

V. SUMMARY OF THE CLAIMED SUBJECT MATTER

In the following summary, references to Appellant's specification disclosure are included in parentheses. The presently claimed invention relates to a black currant anthocyanin-containing food composition with no monosaccharide, whereby anthocyanin is obtained at an unexpected high concentration using a negatively charged reverse osmosis membrane process (Specification, page 19, lines 12-25). Appellant will show that the prior art does not teach, nor is there any

reason or suggestion in the prior art to use, a negatively charged reverse osmosis membrane to produce a highly concentrated anthocyanin-containing food composition containing no monosaccharide that results (Specification, Figure 2) in unexpected improvements in asthenopia, a visual function relating to adaptation to darkness, blood fluidity and blood pressure as claimed.

Appellant's claimed invention for a highly concentrated anthocyanin-containing food composition from the black currant is accompanied by sugar, organic acids, and the like being removed at the same time as water using the negatively charged reverse osmosis membrane. (Specification page 19, lines 12-25.) The high concentrated anthocyanin-containing composition has no monosaccharide, is low in acidity and sweetness so that the flavor can be incorporated into any type of food, and is thus an excellent starting material for foods (Specification pages 20, lines 1-4; Specification page 32, line 10 stating "*Monosaccharide was not detected at all*"). Furthermore, anthocyanin obtained from black currant using a negatively charged reverse osmosis membrane is excellent in stability unlike conventional fruit juice (Specification pages 48-49, Example 13).

Appellant reports in Examples 3 - 12 (Specification page 34, line 23 to page 48, line 3 and Figure 2) on the experiments that show measurable improvements from the ingestion of the presently claimed invention in eye and vision problems, improving blood fluidity and health related problems resulting therefrom, and improving blood pressure.

The presently claimed invention contains two independent claims 27 and 29, and dependent claims 35-40 and 49 are composition claims. Dependent claims 30 and 32 - 34 are process claims and 44 is a product-by-process claim. Dependent claims 35-40 state common

limitations relating to food consumption and defining improvements in alleviating asthenopia that improves visual adaptation to darkness, and improving blood fluidity that lowers blood pressure.

Independent Claim 27

Independent claim 27 defines a black currant anthocyanin-containing food composition suitable for human consumption (Specification page 1, first paragraph), which comprises by weight,

- (a) 5 to 25% of black currant anthocyanin (Specification page 16, first paragraph), and
- (b) an organic acid content of not more than 5% on the basis of solid matters, monosaccharide is not found (Specification page 19, line 29, page 20, line 1, and page 32, line 10).

Dependent Claim 28

Dependent claim 28 depends from independent claim 27 and recites the limitation that the black currant anthocyanin comprises delphinidin in an amount of 2.5 to 12.5 % by weight on the basis of solid matters (Specification, page 16, lines 6-9).

Independent Claim 29

Independent claim 29 defines a black currant anthocyanin-containing food composition suitable for human consumption (Specification page 1, first paragraph), which comprises by weight,

(a) 5 to 25% of black currant anthocyanin (Specification page 16, first paragraph), and

(b) an organic acid content of not more than 5% on the basis of solid matters, monosaccharide is not found (Specification page 19, line 29, page 20, line 1, and page 32, line 10)

In addition, independent claim 29 defines:

(c) wherein black currant anthocyanin comprises delphinidin-3-o-rutinoside in an amount of 2 to 10 % by weight on the basis of solid matters (Specification page 16, lines 10-13).

Dependent Claim 30

Dependent claim 30 is a process claim that depends from independent claim 27. Claim 30 defines a negatively charged reverse osmosis membrane to produce a purified and concentrated black currant anthocyanin-containing food composition (Specification, page 16, lines 14-18; page 19, lines 12-25).

Dependent Claims 32 - 34

Dependent claims 32 and 33 are process claims that depend from independent claim 30 and further define a negatively charged reverse osmosis membrane; namely, claim 32 further defines a salt retention rate of 5 to 20% in the case of NaCl, and claim 33 further defines an ion-exchange resin that is used to adsorb and concentrate anthocyanin (Specification, page 16, lines 14-19).

Dependent claim 34 is a process claim that depends on dependent claim 33 and further defines the ion-exchange resin is a strong acid cation-exchange resin (Specification, page 16, lines 20-24).

Dependent Claims 35-40

Dependent claim 35 depends from independent claim 27 and defines a food or drink; claim 36 and 38 depend from claim 35, where claim 36 defines the food or drink as candy, chewing gum, juice, chocolate, table, gelatinous food, or jam and claim 38 states the improved visual function of alleviating asthenopia compared to asthenopia before ingestion of the composition and similarly as improving adaptation to darkness (Specification, page 16, lines 25-29; page 17, lines 1-5; and Figure 2).

Dependent claim 37 depends from independent claim 27 and states the improved visual function of alleviating asthenopia compared to asthenopia before ingestion of the composition

and similarly as improving adaptation to darkness (Specification, page 17, lines 1-5; page 28, lines 2-15; and Figure 2).

Dependent claim 39 depends from independent claim 27 and stated the effect for improving blood fluidity compared to blood fluidity before ingestion of the composition and/or an effect for lowering blood pressure compared to blood pressure before ingestion of the composition (Specification, page 17, lines 6-8; page 29, lines 19-29; page 30, lines 1-5).

Dependent claim 40 depends from the food or drink according to dependent claim 35 and which has one of an effect similarly to dependent claim 39 for improving blood fluidity and blood pressure (Specification, page 17, lines 6-8).

Dependent Claim 44

Dependent claim 44 is a product-by-process claim that depends from independent claim 27 and further defines the anthocyanin-containing food composition suitable for human consumption, which is prepared by purifying, separating and concentrating the black currant anthocyanin in a retentate with a negatively charged reverse osmosis membrane from monosaccharides and acids contained in a black currant raw material (Specification, page 19, lines 12-25).

Dependent Claim 49

Dependent claim 49 depends from independent claim 27 and further defines a form of a member selected from the group consisting of a paste, gel and powder (Specification, page 21, lines 4-6).

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

Did the Examiner err in rejecting claims 27-30, 32-40, 44, and 49 under 35 U.S.C. §103(a) are unpatentable over Nakhmedov *et al.* (1975) (Konservnaya I Ovoshchesushil'naya Promyshlennost, hereinafter referred to as "Nakhmedov") in view of U.S. Patent No. 4,643,902 of Lawhon *et al.* ("Lawhon") and Laboratoires Chibret, British Patent Specification No. 1,007,751 ("British '751")?

VII. ARGUMENT

Executive Summary of Appellant's Arguments

- (1) **No reference cited in the rejection of Appellants' claims teaches or suggests a black currant anthocyanin-containing food composition suitable for human consumption, which comprises 5 to 25 % by weight of black currant anthocyanin and an organic acid content of not more than 5 % by weight on the basis of solid matters, and no monosaccharide, as required in all claims on appeal.**
 - (1)(a) **Nakhmedov does not teach or suggest a black currant anthocyanin-containing food composition suitable for human consumption, which comprises an organic acid content of not more than 5 % by weight.**
 - (1)(b) **Nakhmedov does not teach or suggest a black currant anthocyanin-containing food composition suitable for human consumption, which comprises no monosaccharide.**
 - (1)(c) **The Examiner's erred by explaining that Nakhmedov teaches a black currant anthocyanin-containing food composition suitable for human consumption, which comprises an organic acid content of not more than 5 % by weight and no monosaccharide is contrary to the understanding and knowledge of one skilled in the art.**
 - (1)(d) **The Examiner's erred by interpreting the language "food" and "drink" in claims 27-29, 35-40 and 49 on appeal as intended use.**
 - (1)(e) **The Examiner has not provided a sufficient reason for one of ordinary skill in the art to modify a composition, alleged based on the combination of Nakhmedov, Lawhorn, and British '751 to a black currant anthocyanin-containing food composition suitable for human consumption, which comprises 5 to 25 % by weight of black currant anthocyanin and an organic acid content of not more than 5 % by weight on the basis of solid matters, and no monosaccharide, as required in all claims on appeal.**
- (2) **The combined teachings of Nakhmedov, Lawhorn, and British '751 do not contemplate a process for producing a black currant anthocyanin-containing food composition suitable for human consumption, which comprises 5 to 25**

% by weight of black currant anthocyanin and an organic acid content of not more than 5 % by weight on the basis of solid matters, and no monosaccharide, wherein black currant juice, as a starting material is purified and concentrated with a “negatively charged reverse osmosis membrane” as required in claims 30, 32-34, and 44 on appeal.

(3) **Product-by-process claim 44 on appeal defines a composition that is patently distinguishable from the prior art, especially any compositions taught by the combined teachings of Nakhmedov, Lawhorn, and British ‘751.**

Appellant’s Arguments

(1) **No reference cited in the rejection of Appellants’ claims teaches or suggests a black currant anthocyanin-containing food composition suitable for human consumption, which comprises 5 to 25 % by weight of black currant anthocyanin and an organic acid content of not more than 5 % by weight on the basis of solid matters, and no monosaccharide, as required in all claims on appeal.**

(1)(a) **Nakhmedov does not teach or suggest a black currant anthocyanin-containing food composition suitable for human consumption, which comprises an organic acid content of not more than 5 % by weight.**

The Final Office Action, page 2, last paragraph stated that Nakhmedov encompasses a 0% organic acid. This position is in error, and the Examiner’s error is shown in the more detailed analysis in Table 3 of Nakhmedov, which includes monosaccharide content and acidity of the coloring agent -- the data in Tables 1 and 3 of Nakhmedov is for the same marks. In Table 3, Nakhmedov reports the percentage of total acidity for colors in mg per 100 for fresh black currant marc to be $9.8 \pm 0.2 \%$ and for molded black currant marc to be $5.4 \pm 0.2 \%$. In other words, the ranges of Nakhmedov for total acidity for fresh black currant marc is 9.6% to

10% and for molded black currant marc is 5.2% to 5.6%, which is outside the range of Appellant's claimed organic acid content of not more than 5 % by weight.

Appellant respectfully submits that Table 3 of Nakhmedov also reports the parameters on a "molded" coloring agent, which cannot be compared with Appellant's claimed composition since the "molded" composition is affected by bacteria and/or fungus. At least for this reason, such a molded composition would not be considered a food or drink composition (as required in claims 27-29, 35-40, 44, and 49 on appeal) by one of ordinary skill in the art.

From the above, Appellant respectfully submits that it is incontrovertible that the marcs in Table 1 as stated in Table 3 of Nakhmedov necessarily contain amounts of organic acids in excess of that required in the present claims.

(1)(b) Nakhmedov does not teach or suggest a black currant anthocyanin-containing food composition suitable for human consumption, which comprises no monosaccharide.

The Final Office Action, page 2, last paragraph, commented that Nakhmedov teaches in Table 1, a black currant composition comprising 6256.8 ± 11.5 mg/100 g and 6128.9 ± 15.2 mg/100 g, which corresponds to approximately 6.26% that is encompassed by the presently claimed amount of 5 to 25% of black currant anthocyanin, and such a composition meets the requirements of applicant's claim because there is no monosaccharide contained therein and there is no organic acid mentioned therein.

The Examiner errs by misinterpreting the teachings of Nakhmedov and reads into the reference the nonexistence of monosaccharide and organic acid when in fact Nakhmedov reports

no such information. As discussed in Section (1)(a) above, the result of a more detailed analysis including the content of monosaccharide and acidity of the coloring agent is disclosed in Table 3 -- this data is for the same marks as in Table 1 of Nakhmedov. In Table 3, Nakhmedov reports the percentage of total acidity ranges of 5.2 to 10% in mg per 100; the percentage of sugar content in mg per 100 reports 16.1 ± 0.7 for colors from fresh black currant marc and 13.3 ± 0.8 % for colors from molded black currant marc.

Appellant respectfully submits that Table 3 of Nakhmedov also reports the parameters on a “molded” coloring agent, which cannot be compared with Appellant’s claimed composition since the “molded” composition is affected by bacteria. Furthermore, such a molded composition would not be considered a food composition (as required in claims 27-29, 35-40, 44, and 49 on appeal) by one of ordinary skill in the art.

Appellant maintains a patentable distinction between the presently claimed invention and the teachings of Nakhmedov in that Nakhmedov includes monosaccharide in the compositions proposed therein, while Appellant’s claims exclude the presence of monosaccharide.

(1)(c) The Examiner’s erred by explaining that Nakhmedov teaches a black currant anthocyanin-containing food composition suitable for human consumption, which comprises an organic acid content of not more than 5 % by weight and no monosaccharide is contrary to the understanding and knowledge of one skilled in the art.

It is well known in the scientific community and by one skilled in the art that fruit, including the black currant fruit necessarily contains organic acids and monosaccharides. The teachings of Nakhmedov do teach or suggest no monosaccharide and/or low acidity for a black currant anthocyanin-containing food composition suitable for human consumption.

The Final Office Action alleged that the sugar content shown in Table 3 of Nakhmedov can be monosaccharides, disaccharides and complex carbohydrates and concluded that absent evidence to the contrary, Nakhmedov teaches a sugar, not a monosaccharide. While not believed necessary, Appellant submitted evidence establishing that any person skilled in this art would know or easily determine by simple procedures that the sugars shown in Table 3 of Nakhmedov necessarily contain a monosaccharide.

This conclusion is supported by the article by Boccorh *et al.* (hereinafter referred to as Boccorh), entitled *Factors influencing quantities of sugars and organic acids and blackcurrant concentrates* in *Z Lebensm Unters Forsch A* (1998) 206; 273-278, which discloses the sugar contents of black currant concentrate in Table 1 thereof. Table 1 clearly shows that the main sugars of black currant concentrate are fructose and glucose -- monosaccharides. Additionally, Appellant presented an article of Sanna Viljakainen (hereinafter referred to as “Sanna”), entitled *Reduction of Acidity in Northern Region Berry Juices* in ISBN 951-22-6435-8 (2003). From this information, those skilled in the art would understand that the composition of marcs disclosed in Table 1 of Nakhmedov must necessarily contain amounts of organic acids and monosaccharides, in excess of that required in the claims on appeal.

In the Final Office Action, the Examiner states that Appellant’s reliance on Sanna is not persuasive since the determination of obviousness or nonobviousness must be based upon what was known in the art at the time the invention was made. Examiner’s reasoning is scientifically flawed. Sanna states that black currant juice contains sugar and organic acids. The black currant at the time of the present invention is no different than that of centuries past. The publication

date of Sanna is not pertinent to the issue here. For example, it is common for applicants to submit affidavits demonstrating patentability of a claimed invention. The fact that the affidavit is submitted after the filing date of the invention does not affect its materiality. The same is true of the Sanna article.

Table 1 of Nakhmedov was created only to show limited information therein, such as only the anthocyanin and coloring agent content in marcs, and not to obscure this showing by providing additional information containing organic acids, monosaccharides, etc. Black currant necessarily contains organic acids and monosaccharides, as understood by any person skilled in the art and as taught by Nakhmedov. Table 3 of Nakhmedov shows *the same black currant marcs* have $9.8 \pm 0.2\%$ of total acidity. Moreover, Sanna discusses that the main acids of black currant berry juices were invariably citric and malic acid. Furthermore, those skilled in the art understand that the total acidity means the content of organic acids, because Nakhmedov refers to the content of organic acids and sugars in the marc at the 9th line from the bottom on page 6 to the 6th line from the bottom on page 6 of the English translation of Nakhmedov.

Furthermore, Table 3 of Nakhmedov also reports the parameters on a “molded” coloring agent, which cannot be compared with Appellant’s claimed composition since the “molded” composition is affected by bacteria. A molded composition would not be considered a food composition (as presently claimed) by one of ordinary skill in the art.

Appellant maintains a patentable distinction between the presently claimed invention and the teachings of Nakhmedov in that Nakhmedov includes monosaccharide in the compositions proposed therein, while Appellant’s claims exclude the presence of monosaccharide.

Appellant respectfully submits that it is impermissible within the framework of 35 U.S.C. §103 to select a single line or two of a reference (i.e., only Table 1 of Nakhmedov) in total disregard for the remaining teachings of the reference (i.e., Table 3 of Nakhmedov) and then rely upon the reference with the benefit of hindsight to show obviousness. *Bausch & Lomb, Inc. v. Barnes-Hind/Hydrocurve, Inc.*, 230 U.S.P.Q. 416, 419 (CAFC 1986). It has long been held that it is impermissible within the framework of §103 to pick and choose from any one reference only so much of it as will support a given position to the exclusion of other parts necessary to the full appreciation of what such reference fairly suggests to one skilled in the art. *In re Wesslau*, 147 U.S.P.Q. 391, 393 (CCPA 1965); *In re Mercer*, 185 U.S.P.Q. 774, 778 (CCPA 1975). For these reasons, Appellant respectfully submits that the teachings of Nakhmedov either alone or combined with Lawhon and British '751 cannot contemplate or suggest a composition containing a limited amount of organic acid and no monosaccharide, as presently claimed and do not establish a *prima facie* case of obviousness for the inventions claimed on appeal within the meaning of 35 U.S.C. §103(a).

(1)(d) The Examiner's erred by interpreting the language "food" and "drink" in claims 27-29, 35-40 and 49 on appeal as intended use.

The claims in the present application plainly define a food composition or drink suitable for human consumption. This claim language is not *intended use* but pertains to the structure of the composition. There is no reasonable expectation that a coloring agent, such as that proposed by Nakhmedov, can be used in a food or drink suitable for human consumption. For example, the U.S. Food & Drug Administration has stringent guidelines for coloring agents used in foods

and drink. One of ordinary skill in the art would expect that a composition, such as that proposed by Nakhmedov, which has high acid content and/or is moldy, would not be suitable as a food or drink. Therefore, Nakhmedov cannot disclose or suggest the invention claimed on appeal.

The teachings of Nakhmedov cannot disclose or suggest the inventions defined in, for example, claim 27 that are directed to "A black currant anthocyanin-containing *food composition suitable for human consumption*." The Final Office Action provided no reasons why the arguments provided in the response are not correct, but simply stated that the marks of Nakhmedov are capable performing the intended use of the present claims. Appellant respectfully submits that this position is incorrect for least the following reasons.

The teachings of Lawhon are not concerned with concentrating black currant juices and the methods proposed therein cannot obtain a composition having anthocyanin, organic acid and monosaccharide concentrations as presently claimed. As discussed above, the teachings of Nakhmedov are concerned with preparing a coloring agent. Since the teachings of Lawhon and Nakhmedov have nothing to do with a "black currant anthocyanin-containing *food composition suitable for human consumption*," and, in fact, teach away from such a composition, Appellant respectfully submits that these teachings either alone or combined with other teachings cannot provide any reason to one of ordinary skill in the art prepare a "black currant anthocyanin-containing *food composition suitable for human consumption*," as presently claimed.

None of the cited teachings contemplates or suggests the amount of delphinidin and delphinidin-3-O-rutinoside, required for example, in present claims 28 and 29, together with the other requirements of these claims. While the presence of delphinidin or delphinidin-3-O-

rutinoside may have been known in black currant, which the Appellant does not admit, an important characteristic of the presently claimed invention is a composition containing a high content of delphinidin and delphinidin-3-O-rutinoside as a food composition (i.e., having low amounts of monosaccharides and organic acids). The teachings of Nakhemedov are concerned with obtaining a dye. In contrast, the presently claimed invention is directed to obtaining a food composition (substantially free of monosaccharide and having low content of organic acid). Therefore, Appellant respectfully submits that the teachings of Nakhemedov can provide a person of ordinary skill in the art with no reason to obtain the presently claimed food composition. Furthermore, Nakhemedov never suggests the content of delphinidin-3-O-rutinoside, and therefore cannot contemplate or suggest the presently claimed amount of delphinidin-3-O-rutinoside, etc.

With respect to present claims 35 and 36, the presently claimed food or drink includes the black currant anthocyanin-containing food composition according to claim 27. As explained above, the black currant anthocyanin-containing food composition according to claim 27 has structural differences from the prior art. For at least this reason, the presently claimed food or drink of claims 35 and 36 is patently distinguishable from the prior art.

Concerning present claims 37 to 40, the claimed black currant anthocyanin-containing food composition according to claim 27. Claims 37 and 39 that depend from claim 27 are directed to a food composition. As mentioned above, the claimed composition of claim 27 is different from the composition disclosed in Nakhmedov, Lawhon, and British '751. For at least this reason, the presently claimed food or drink of claims 37 and 39 is patently distinguishable

from the prior art.

Present claims 38 and 40 depend from claim 35, which depends on claim 27, and are directed to a food or drink. As mentioned above, the claimed composition of claim 27 is different from the composition disclosed in Nakhmedov, Lawhon, and British '751. For at least this reason, the presently claimed food or drink of claims 38 and 40 is patently distinguishable from the prior art.

Furthermore, the food composition of present claims 37 and 39, and the food or drink of present claims 38 and 40 contain anthocyanin amount that accomplishes the properties recited in each claim. That is, the properties recited in present claims 37 to 40 patently distinguish the claimed composition and food or drink from the different composition proposed by Nakhmedov, Lawhon, and British '751. For least this reason, the presently claimed food compositions of claims 27-29, 37, 39 and 49, and the food and drink compositions of claims 35, 36, 38 and 40 are clearly different from the composition disclosed in Nakhmedov, Lawhon, and British '751 and do not establish a *prima facie* case of obviousness for the invention claimed on appeal within the meaning of 35 U.S.C. §103(a).

(1)(e) The Examiner has not provided a sufficient reason for one of ordinary skill in the art to modify a composition, alleged based on the combination of Nakhmedov, Lawhorn, and British '751 to a black currant anthocyanin-containing food composition suitable for human consumption, which comprises 5 to 25 % by weight of black currant anthocyanin and an organic acid content of not more than 5 % by weight on the basis of solid matters, and no monosaccharide, as required in all claims on appeal.

The coloring agent defined in the teachings of Nakhmedov is not a food. The attached scheme, which is labeled as "Attachment 1," shows the process of purifying a coloring agent proposed in Nakhmedov. Table 1 of Nakhmedov was created only to show the information therein, such as only that anthocyanin and coloring agent content in marc. Reading information relating to the nonexistence of monosaccharide and/or organic acids is to obscure the teachings of Nakhmedov.

In the Nakhmedov process, as shown in Attachment 2, the raw material is pressed and a part other than juice is used as pressed skin, which is usually treated as a waste, to purify the coloring agent. The pressed skin is divided into two parts. The anthocyanin content of the first part of the pressed skin is analyzed as the firstly pressed marc and the result is disclosed in Table 1 (Contents in marcs, of the first pressing). The second part of the pressed skin is further pressed and analyzed as the secondarily pressed marc and the result is disclosed in Table 1 (Content in marcs, of the second pressing). The firstly pressed marc and secondarily pressed marc are further treated to separate juice, and the coloring agent is extracted with hot water. The anthocyanin content of the coloring agent from the firstly pressed mark is disclosed in Table 1 (Quantity of

coloring agent produced from marc, of the first pressing). The anthocyanin content of the coloring agent from the secondarily pressed mark is also disclosed in Table 1 (Quantity of coloring agent produced from marc, of the second pressing). The result of detailed analysis including the content of monosaccharide and acidity of the coloring agent is disclosed in Table 3 -- this data is for the same marks as in Table 1 of Nakhmedov.

Appellant respectfully submits that the currently claimed invention is patentable distinct from Nakhmedov's composition for a coloring agent. Tables 1 and 3 of Nakhmedov show the analytical data of colors from black currant marc. Table 1 only reports on the content of anthocyanin in marcs, which is the meaning of Table 1. Table 3 of Nakhmedov reports on the content of several parameters of the coloring agent including sugar content of 16.1% and not free of monosaccharide (i.e., 0%) as currently defined in Appellant's claimed invention. Table 3 of Nakhmedov also reports the parameters on a "molded" coloring agent, which cannot be compared with Appellant's claimed composition since the "molded" composition is affected by bacteria. Furthermore, such a molded composition would not be considered a food composition (as presently claimed) by one of ordinary skill in the art.

Appellant maintains a patentable distinction between the presently claimed invention and the teachings of Nakhmedov in that Nakhmedov includes monosaccharide in the compositions proposed therein, while Appellant's claims exclude the presence of monosaccharide.

(2) **The combined teachings of Nakhmedov, Lawhorn, and British '751 do not contemplate a process for producing a black currant anthocyanin-containing food composition suitable for human consumption, which comprises 5 to 25 % by weight of black currant anthocyanin and an organic acid content of not more than 5 % by weight on the basis of solid matters, and no monosaccharide, wherein black currant juice, as a starting material is purified and concentrated with a “negatively charged reverse osmosis membrane” as required in all claims 30, 32-34, and 44 on appeal.**

Appellant's claimed invention is a black currant anthocyanin-containing food composition suitable for human consumption that unexpectedly contains greater amounts of anthocyanin obtained by a “negatively charged reverse osmosis” that eliminates monosaccharide and produces low organic acid content. The stability of the claimed composition is superior due to the absence of monosaccharide and low organic acid content, because it is well known that monosaccharide and organic acids lead to the decomposition of foods. The graph in Figure 2 of specification demonstrates the effects before and after ingestion of the claimed composition in effectively alleviating asthenopia or adaptation to darkness. Another benefit of the claimed invention is increasing blood fluidity with the effect of lowering blood pressure.

On page 3, lines 14-15, the examiner incorrectly states claims 30, 32, 33, and 34 as product-by-process claims, when these claims are process claims. Accordingly, the comments in the Final Office Action concerning product-by-process claims are not pertinent to these claims. Appellant respectfully submits that claims 30, 32, 33, and 34 are patently distinguishable from the teachings of Nakhmedov, Lawhon, and British '751 within the meaning of 35 U.S.C. §103 for at least the following reasons.

Page 9, lines 3-12 of the Final Office Action states that:

Although Lawhon does not specifically recite "a charged reverse osmosis (RO) membrane, example 4 in column 10 recites that the RO membrane system is stated to have a 99% rejection for NaCl. It is well known in the art that reverse osmosis is capable of rejecting bacteria, salts, sugars, proteins, particles, dyes and other constituents that have a molecular weight of greater than 150 to 250 Daltons. The separation of ions with reverse osmosis is aided by charged particles. This means that dissolved ions that carry a charge, such as salts, are more likely to be rejected by the membrane than those that are not charged, such as organics. The larger the charge and the larger the particles, the more likely it will be rejected. Since the invention of Lawhon rejects 99% NaCl, the RO membrane is aided by charged particles.

Appellant argues that the teachings of Lawhon propose the purification and concentration of juice by using a reverse osmosis membrane. In the process disclosed in Lawhon using reverse osmosis (RO) membrane, sugars and acids are fractionated in a RO retentate together with anthocyanin. In contrast thereto, the membrane used for the presently claimed invention is a *negatively charged* reverse osmosis membrane. In the process of the presently claimed invention using the *negatively charged* RO membrane, anthocyanin is fractionated in a retentate however sugars and acids are separately fractionated in a permeate -- an arrangement opposite to that proposed by Lawhon.

The RO membrane, such as proposed by Lawhon, was developed for desalinating seawater to obtain fresh water. The RO membrane is placed between seawater and fresh water. When a pressure higher than the pressure of osmotic pressure is applied to seawater, water in the seawater is moved to the fresh water through the RO membrane. Since the retention rate of NaCl of the RO membrane is more than 99%, fresh water can be obtained by using the RO membrane.

In contrast thereto, the charged RO membrane was developed separately from the RO membrane as a nanofilter (or a loose RO membrane). The size of a substance removed by the charged RO membrane is between that of the RO membrane and ultrafiltration (UF) membrane. That is, the charged RO membrane operates and functions differently from a RO membrane as a filter, as understood by those skilled in the art.

Therefore, the teachings of Lawhon cannot possibly provide a reason to one of ordinary skill in the art to use a process proposed therein in combination with a negatively charged reverse osmosis membrane, as required in present claim 30. In fact, the use of a negatively charged reverse osmosis membrane in the process claims on appeal provides an *unexpectedly superior* process and composition having large amounts of anthocyanin, while having low amounts of organic acids and no monosaccharide, as required in the claims on appeal. The teachings of Nakhmedov and British '751 do not cure or rectify these deficiencies in the teachings of Lawhon. At least for these reasons, Appellant respectfully submits that the invention defined in independent process claim 30 and the claims that depend thereon are patently distinguishable from the teachings of Nakhmedov, Lawhon, and British '751.

(3) Product-by-process claim 44 on appeal defines a composition that is patently distinguishable from the prior art, especially any compositions taught by the combined teachings of Nakhmedov, Lawhorn, and British '751.

On page 7, lines 5-9 of the Final Office Action, the Examiner states that "*it would have been obvious to produce a black currant anthocyanin composition in which monosaccharide "is not found". Such a modification would have been motivated by the reasoned expectation of*

producing a black currant anthocyanin composition which is effective in comprehensively treating persons suffering from dental caries, diabetes or obesity where the presence is a saccharide is detrimental." The Examiner has not established a factual basis of a fruit juice such as the black currant fruit juice having no monosaccharide, which is necessary to support the reasons to modify the composition allegedly proposed by the combined teachings of Nakhmedov, Lawhorn, and British '751. In other words, Appellant argues that the Examiner is erroneously relying on hindsight to establish a reason to modify the alleged prior art composition to that defined in the claims on appeal, which is impermissible.

Claim 44 is a product-by-process claim. Appellant respectfully submits that the present specification disclosure establishes the importance and significance of the method steps set forth in claim 44, and moreover, establishes that the product resulting from the product-by-process steps set forth therein results in a patently distinguishable product. Namely, in the presently claimed invention, the organic acids content can be reduced without using an ion exchange column.

Example 1 of the present application shows that the use of the negatively charged reverse osmosis membrane itself accomplished the reduction of organic acids and the deletion of monosaccharide. As shown in Example 2 of the present application, the ion exchange column was used to increase anthocyanin content, not to reduce the organic acids content. These examples show the differences between the use of a negatively charged reverse osmosis membrane and an ion exchange column, especially with respect to the presently claimed invention, and that they cannot be interchangeably used to arrive at the compositions defined in

the present claims.

For such reasons, Appellant respectfully submits that the invention defined in claim 44 is patentably distinguishable from the teachings of Nakhmedov, Lawhon, and British '751 and do not establish a *prima facie* case of obviousness for the inventions claimed on appeal within the meaning of 35 U.S.C. §103(a).

In summary, the Examiner apparently took the position that Nakhmedov teaches the amounts of solids left from processing black currant berries, as proposed therein, and combining this knowledge with the process as proposed by Lawhon will lead to Appellant's claimed invention. However, as explained above, even if one of ordinary skill in the art did this, the teachings of Nakhmedov provide no reason to one of ordinary skill in the art to modify the process proposed by Lawhon so as to arrive at the presently claimed compositions that have specific amounts of anthocyanin, organic acid, and no monosaccharide.

The bottom paragraph of page 7 of the Final Office Action stated that one cannot show nonobviousness by attacking references individually where the rejections are based on combination of references. Appellant has consistently argued, including throughout Appellant's appeal brief presented herewith, that the claimed invention is patentably distinguishable from the combined teachings of the references cited including Nakhmedov combined with Lawhon and British '751.

The teachings of Nakhmedov are not concerned with concentrating fruit juice. Namely, the teachings of Nakhmedov are directed to the production of coloring agent from marcs (waste) of black currant. The teachings of Nakhmedov never contemplate or suggest concentrating fruit

juice, as presently claimed. An important aspect of the presently claimed invention is reducing the concentration of monosaccharide and organic acids in a composition made from black currant. The teachings of Nakhmedov never suggest the reduction of monosaccharide and organic acids. Therefore, the teachings of Nakhmedov can provide no reason to those skilled in the art to modify the process of Lawhon so as to arrive at the presently claimed composition that have specific amounts of anthocyanin, organic acid, and no monosaccharide.

Furthermore, the teachings of Nakhmedov never disclose or suggest the presently claimed composition. Similarly, the teachings of Lawhon never disclose or suggest the claimed process of the present application. The teachings of British '751 do not cure or rectify these deficiencies in the teachings of Nakhmedov and Lawhon. Accordingly, it is impossible for one of ordinary skill in the art to review the combined teachings of Lawhon, Nakhmedov, and British '751 and have a reason to prepare the compositions of Appellant's claims. For at least this reason, Appellant respectfully submits that the inventions defined in claims 27-30, 32-40, 44 and 49 are patently distinguishable from the combined teachings of Lawhon, Nakhmedov, and British '751 within the meaning of 35 U.S.C. §103 and do not establish a *prima facie* case of obviousness for the inventions claimed on appeal within the meaning of 35 U.S.C. §103(a).

CONCLUSION

For at least the foregoing reasons Appellant respectfully submits that the present claims are patently distinguishable from the teachings of Lawhon, Nakhmedov, and/or British '751 within the meaning of 35 U.S.C. §102 or 35 U.S.C. §103. Therefore, it is respectfully requested that this honorable Board reverse the rejection of claims 27-30, 32-40, 44, and 49 under 35 U.S.C. § 103(a) as being unpatentable over Lawhon, Nakhmedov, and/or British '751.

Respectfully submitted,
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VIII. CLAIMS APPENDIX

27. A black currant anthocyanin-containing food composition suitable for human consumption, which comprises 5 to 25 % by weight of black currant anthocyanin and an organic acid content of not more than 5 % by weight on the basis of solid matters, and monosaccharide is not found.

28. The black currant anthocyanin-containing food composition suitable for human consumption according to claim 27, wherein the black currant anthocyanin comprises delphinidin in an amount of 2.5 to 12.5 % by weight on the basis of solid matters.

29. The black currant anthocyanin-containing food composition suitable for human consumption, which comprises 5 to 25 % by weight of black currant anthocyanin and an organic acid content of not more than 5 % by weight on the basis of solid matters, and monosaccharide is not found, wherein black currant anthocyanin comprises delphinidin-3-o-rutinoside in an amount of 2 to 10 % by weight on the basis of solid matters.

30. A process for producing black currant anthocyanin-containing food composition suitable for human consumption according to claim 27; wherein black currant juice, as a starting material, is purified and concentrated with a negatively charged reverse osmosis membrane.

32. The process for producing black currant anthocyanin-containing food composition suitable for human consumption according to claim 30, wherein the negatively charged reverse osmosis membrane has a salt retention rate of 5 to 20% in the case of NaCl.

33. The process for producing black currant anthocyanin-containing food composition suitable for human consumption according to claim 30, wherein an ion-exchange resin is also used to adsorb and concentrate anthocyanin.

34. The process for producing black currant anthocyanin-containing food composition suitable for human consumption according to claim 33, wherein the ion-exchange resin is a strong acid cation-exchange resin.

35. A food or drink including the black currant anthocyanin-containing food composition suitable for human consumption according to claim 27.

36. The food or drink according to claim 35; wherein the food or drink is candy, chewing gum, juice, chocolate, tablet, gelatinous food, or jam.

37. The black currant anthocyanin-containing food composition suitable for human consumption according to claim 27, which contains an effective amount of the black currant anthocyanin for improving visual function selected from the group consisting of alleviating asthenopia compared to asthenopia before ingestion of the composition and improving adaptation to darkness as compared to adaptation to darkness before ingestion of the composition.

38. The food or drink according to claim 35, which contains an effective amount of the black currant anthocyanin for improving visual function selected from the group consisting of alleviating asthenopia compared to asthenopia before ingestion of the composition and improving adaptation to darkness as compared to adaptation to darkness before ingestion of the composition.

39. The black currant anthocyanin-containing food composition suitable for human consumption according to claim 27, which has an effect for improving blood fluidity compared to blood fluidity before ingestion of the composition and/or an effect for lowering blood pressure compared to blood pressure before ingestion of the composition.

40. The food or drink according to claim 35, which has at least one of an effect for improving blood fluidity compared to blood fluidity before ingestion of the composition and an effect for lowering blood pressure compared to blood pressure before ingestion of the composition.

44. The black currant anthocyanin-containing food composition suitable for human consumption according to claim 27, which is prepared by purifying, separating and concentrating the black currant anthocyanin in a retentate with a negatively charged reverse osmosis membrane from monosaccharides and acids contained in a black currant raw material.

49. A black currant anthocyanin-containing food composition suitable for human consumption according to claim 27 further processed into a form of a member selected from the group consisting of a paste, gel and powder.

IX. EVIDENCE APPENDIX

Appellant hereby attaches an Evidence Appendix containing the following document discussed in the denoted pages of Appellant's brief.

Attachment 1: English Abstract and English Translation of Nakhmedov (10 pages). (English Abstract cited by Examiner in Office Action dated September 9, 2005; English translation submitted by Appellant in a Voluntary Amendment dated February 10, 2005)

Attachment 2: Scheme showing the process of purifying a coloring agent proposed in Nakhmedov (one page). (Filed by Appellant in a Response filed on November 5, 2007)

Attachment 3: Boccorh *et al.*, entitled *Factors influencing quantities of sugars and organic acids and blackcurrant concentrates* in Z Lebensm Unters Forsch A (1998) 206; 273-278 (six pages). (Filed by Appellant as Attachment 2 in a Response filed on November 5, 2007)

Attachment 4: Sanna Viljakainen, entitled *Reduction of Acidity in Northern Region Berry Juices* in ISBN 951-22-6435-8 (2003) (four pages). (Filed by Appellant as Attachment 3 in a Response filed on November 5, 2007)

X. RELATED PROCEEDING APPENDIX

Appellant states that there is no related proceeding.

of 520 mgs. per liter. The retentate can be dried or concentrated further and used as a coloring agent per se or a source of food solids.

L11 ANSWER 11 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1989:447127 BIOSIS
DOCUMENT NUMBER: BA88:95399
TITLE: MECHANIZED CURRANT HARVEST AND PROBLEMS OF FRUIT QUALITY PART II. CHANGES IN THE QUALITY OF BLACK CURRANT FRUITS HARVESTED MECHANICALLY AND BY HAND DURING SHORT TERM STORAGE.
AUTHOR(S): LENARTOWICZ W; ZBROZCZYK J
CORPORATE SOURCE: INSTYTUT SADOWNICTWA I KWIACIARSTWA, SKIERNIEWICE, POLAND.
SOURCE: PR INST SADOW KWIACIARSTWA SKIERNIEWICACH SER A - PR DOSW ZAKRESU SADOW, (1987 (1988)) 27 (0), 143-154.
CODEN: PSKSDQ. ISSN: 0208-5933.
FILE SEGMENT: BA; OLD
LANGUAGE: Polish
TI MECHANIZED CURRANT HARVEST AND PROBLEMS OF FRUIT QUALITY PART II. CHANGES IN THE QUALITY OF BLACK CURRANT FRUITS HARVESTED MECHANICALLY AND BY HAND DURING SHORT TERM STORAGE.
AB Changes in the **black currant** fruit, harvested mechanically and by hand, were investigated during short term storage in different temperatures. It was found that after . . . K (6.degree. C) than at 289-292.5 K (16-19.5.degree. C). Changes in the content of organic substances as dry matter, soluble **solids**, organic acids and **anthocyanins** were similar in fruits harvested by the two methods. Only ascorbic acid content decreased faster in mechanically harvested fruits. Leakage of **juice** from the defrosted fruits was similar. Changes in the organic substances content were much faster and more pronounced at room . . . temperature. Storage at the temperature of 279 K (6.degree. C) decreased the tempo of these changes. It was shown that **black currant** fruits harvested by either methods preserved their good quality at the temperature 279 K (6.degree. C), for 4 days, and . . .
IT Miscellaneous Descriptors
ANTHOCYANINS ORGANIC ACIDS FUNGAL ROT COLD STORAGE

L11 ANSWER 12 OF 12 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1975:407540 CAPLUS
DOCUMENT NUMBER: 83:7540
TITLE: Pigments from the processing by-products of black rowanberries and black currants
AUTHOR(S): Nakhmedova, F. G.; Frumkin, M. L.; Svistunova, V. A.; Myachin, V. M.
CORPORATE SOURCE: Vses. Nauchno-Issled. Inst. Konservn. Ovoshchesush. Prom., USSR
SOURCE: Konservnaya i Ovoshchesushil'naya Promyshlennost (1975), (4), 15-18
DOCUMENT TYPE: Journal
LANGUAGE: Russian
AB Wastes from **black currant** and **black rowanberry** processing contained 5-10% **solids** and 800-2000 mg % **anthocyanins**. The material was contaminated with microorganisms and spoiled readily. The **rowanberry** waste showed cyanidin, cyanidin 3-glucoside, cyanidin 3,5-diglucoside, and a trace of delphinidin during paper chromatog., while the **currant** material showed cyanidin, delphinidin, cyanidin 3-glucoside, and delphinidin 3-rutinoside. The waste materials were potentially good sources of **food** coloring material.
ST **currant black anthocyanin; rowanberry anthocyanin**
IT **Currant**

(black, pigments of, for food coloring)
 IT **Anthocyanins**
 RL: BIOL (Biological study)
 (of currants and rowanberries, for food coloring)
 IT **Sorbus aucuparia**
 (pigments of fruit of, for food coloring)
 IT 15674-58-5
 RL: BIOL (Biological study)
 (of black currants, food coloring by)
 IT 528-53-0 528-58-5 7084-24-4
 RL: BIOL (Biological study)
 (of currants and rowanberries, for food coloring)
 IT 2611-67-8
 RL: BIOL (Biological study)
 (of rowanberries, food coloring by)

=> FIL STNGUIDE			
COST IN U.S. DOLLARS	SINCE FILE	TOTAL	
	ENTRY	SESSION	
FULL ESTIMATED COST	77.98	78.19	
DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE	TOTAL	
	ENTRY	SESSION	
CA SUBSCRIBER PRICE	-0.65	-0.65	

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 AND TECHNOLOGY CORPORATION, AND FACHINFORMATIONSZENTRUM KARLSRUHE

FILE CONTAINS CURRENT INFORMATION.
 LAST RELOADED: Mar 31, 2003 (20030331/UP).

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 0 BLACKCURRANT
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 CAPLUS, CEN, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, DRUGNL, DRUGU, EMBAL,
 EMBASE, ESBIOBASE, IFIPAT, IPA, JICST-EPLUS, KOSMET, LIFESCI, MEDICONF,
 MEDLINE, NAPRALERT, NLDB, NUTRACEUT, ...' ENTERED AT 15:09:03 ON 05 APR
 2003

L1 8787 S BLACKCURRANT OR BLACK CurrANT OR CurrANT
 L2 668275 S % SOLIDS
 L3 13169 S PERCENT SOLIDS

Nakhmedov F.G., Ph.D. (biology),
Frumkin M.L., Ph.D. (engineering),
Svistunova V.A., senior technician,
The All-Union Research Institute for Canning and Vegetable-Drying
Industry;
Myachin V.M.,
Director of Michurinsk canning combinat

**Coloring agents from the wastes of chokeberry and
black currant processing**

Over a period of years we have been researching
chemico-technological and microbiological characteristics of the
wastage of canned foods of dark-colored fruits and berries, with the
purpose to use them for production of natural colors.

Experiments were carried out in laboratory and semi-plant
conditions during 1971-1973 years with the wastes formed in processing
of chokeberry and black currant. Raw material (35-40 kg of each type),
delivered from the farms of Moscow Region, was separately subjected
to process operations (washing, crushing or rubbing, pressing) on the
laboratory equipment according to generally accepted conditions for
the production of natural juices, juices with pulp and juices for
manufacturing of fruit wines.

After separation of juice, the pressed skins were divided into
two parts: one was left as the marc of the first pressing, and the
second was poured with water and in a few hours pressed again for

receiving secondarily pressed marcs. After sampling for chemical and microbiological analyses, the firstly and secondarily pressed marcs were divided into two parts and used for production of colors: one immediately after separation of juice, another after ten-days storage in unsealed container at room temperature. In both cases colors were obtained by extracting the coloring agents with hot water.

In parallel with the laboratory tests, experiments under the same conditions with manufacturing ones were carried out at Michurinsk canning combinat.

The content of anthocyanins in marcs and colors was determined by the method of J. B. Harborne [1] and M. K. Zeikel [2] modified by authors. Anthocyanins were extracted from the marcs with a solution of 3% hydrochloric acid in ethanol, and from colorants with a solution of 2% hydrochloric acid in ethanol.

The anthocyanins content was determined on an FEK-M photoelectrocolorimeter with green filter and in a cell of 10 mm thick. Calibration curve prepared based on pure malvidine preparation was used for conversion of extinction of the solution to be used.

Composition of the anthocyanins was determined by the methods of descending and two dimensional paper chromatography using the following solvents: n-butanole : acetic acid : water (4 : 1 : 1) and 2%-acetic acid. Dry chromatograms were developed by the aqueous solutions of sodium carbonate and sodium acetate. Anthocyanins were identified by comparison of the developed spots with *Rf* on J. B. Harborne [1]. The content of solid matters and sugars, the total acidity and the pH value were determined by generally accepted methods of chemical analysis.

In microbiological experiments, the total number of bacteria was determined by counting the colonies on beef-peptone agar. Mold fungus and yeast were detected by sowing the wash-out solution on wort agar. The inoculaums were maintained at 28 °C during 3 days. The analyses were carried out before depositing marc for storage and periodically during the whole storage period. Samples of 10-20 g were taken from each type of raw materials.

Similar microbiological experiments were carried out for produced colorants.

Process examinations were carried out periodically five times a year. Chemical analysis of marcs and colors was made with three parallel samples, and microbiological analysis - with five samples. Thus obtained results were processed for calculation of arithmetic mean values.

The experiments indicated that the wastes resulting from chokeberry and black currant processing include a significant amount of solid matters and anthocyanins, which preconditions manufacturing of natural food coloring agents from them (Table 1).

Table 1

Marcs	Marc quantity, kg	Content in marcs		Quantity of coloring agent produced from marc		
		dry substances, %	anthocyanins, mg per 100 g	kg	%	content of anthocyanins in coloring agent, mg per 100 g
From the chokeberry						
of the first pressing	38.5	10.8 ± 0.2	1723.5 ± 15.5	9.82	25.5 ± 1.5	5235.4 ± 15.3
of the second pressing	30.4	6.8 ± 0.4	925.3 ± 14.8	3.5	11.5 ± 0.9	4924 ± 10.0
From the black currant						
of the first pressing	40.0	11.9 ± 0.5	2020.8 ± 26.8	9.95	24.9 ± 0.3	6256.8 ± 11.5
of the second pressing	40.0	5.4 ± 0.3	874.7 ± 26.5	4.1	10.25 ± 2.0	6128.9 ± 15.2

As may be seen from the data in Table 1, the higher is the content of solid matters and anthocyanins in the marc, the higher is the yield of the colorants. In the marc of the first pressing the content of solid matters and anthocyanins is two times higher than in marc of the second pressing. Of the marcs of the first pressing, the yield of colorants containing 40% of solid matters amounts to 24-25%, and, of the marcs of the second pressing, the yield - only to 10-11%.

Microbiological experiments showed that the marcs of first and second pressing are heavily bacterized with various microflora: spore bacillus, yeast, non-spore bacillus of Bact. Herbicola type, Proteus, pigmented and not pigmented coccus, lactobacillus and to lesser extent molds (Table 2).

As seen from the data in Table 2, on the first day of depositing berries in storage, newly pressed juice and marcs were sown with microflora almost equally. However on the third day of storage at room temperature, the juice began fermentation; there appeared

evidences of mold growth on berries, while marcs remained almost unchanged. At the end of storage on the tenth day, there appeared an obvious evidence of spoilage in the berries, intense fermentation in the juice, and only a thin layer of molds on the surface of marcs. At the same time, it was discovered that marcs of black currant got moldy quicker than marcs of chokeberry.

Table 2

Product to be tested	Quantity of microorganisms in 1 g before storage of raw material	
	Total	Including yeast and molds
Chokeberry		
Berries	$3.4 \cdot 10^5$	$2.1 \cdot 10^3$
Juice	$1.5 \cdot 10^6$ *	$1.2 \cdot 10^3$ *
Marc	$2.5 \cdot 10^6$	$2.1 \cdot 10^3$
Black currant		
Berries	$2.1 \cdot 10^6$	$3.1 \cdot 10^4$
Juice	$1.3 \cdot 10^4$ *	$1.1 \cdot 10^4$ *
Marc	$3.4 \cdot 10^6$	$1.5 \cdot 10^2$

* In 1 ml

low pH value

Resistance of the marcs to microbiological spoilage during storage may be probably explained by the fact that they are several times poorer in sugars and other nutrients necessary for growth and development of microorganisms, than berries and juices. Besides, the pH value in the marc is significantly lower (~2.9-3.2) than that of berries and juices (~4.0-4.2). Thereby the growth and development of yeast and molds in the marcs proceed slowly.

It is determined that the most acceptable terms of processing the marc into colorants are the first three days after their production.

The marcs sown with the spontaneous microflora and grown moldy to more than 30%, yield such colorant of dark-brown coloration, boiled flavor (smell of mold) higher pH and lower content of anthocyanins than that of marcs with an inconsiderable extent of bacteria growth (Table 3).

Table 3

Parameters	Colors from chokeberry marc		Colors from black currant marc	
	Fresh	molded	fresh	molded
Content of anthocyanins, mg per 100:	6328.3 ± 18.1	2845.6 ± 14.3	7125.4 ± 19.2	3129.9 ± 11.9
pH	3.5 ± 0.2	5.1 ± 0.5	3.2 ± 0.3	4.8 ± 0.5
Total acidity, %	6.9 ± 0.4	4.8 ± 0.3	9.8 ± 0.2	5.4 ± 0.2
Content of sugars, %	15.3 ± 0.2	12.4 ± 0.4	16.1 ± 0.7	13.3 ± 0.8
Appearance	Thick syrup-like liquid, viscous	Less viscous syrup-like liquid	Thick syrup-like liquid, viscous	Less viscous syrup-like liquid
Taste	Slightly acerbic, peculiar to berries	With foreign flavor and slight bitterness	Sour	Less sour with off-flavour
Odour	Peculiar to berries of chokeberry	Of mold	Pleasant, peculiar to berries of black currant	Of mold
Color	Crimson	Dark brown	Crimson	Dark brown
Bacteria growth with microorganisms, cells in 1 ml	0	1.5 · 10 ²	0	1.5 · 10 ⁴

"Canning and vegetable-drying industry", № 4

As seen from the data in Table 3, in the colors produced from the marc without any sign of spoilage, the content of anthocyanins, organic acids and sugars is almost two times higher than in colors from the molded raw materials. This may be explained presumably by the fact that yeast and molds, in order to maintain their viability and intense generation, consume not only sugars and organic acid, but also anthocyanin pigments. Also, the decrease of anthocyanins in the colors from strongly molded marcs is probably induced by the increase of pH value, since when pH value is changed, there occur rearrangement

in anthocyanins structure and formation of free aglycone and of the residues of sugars or new forms of phenolic compounds.

Thus, from the marcs sown with a lot of microorganisms and showing any sign of spoilage, it is impossible to obtain such a food color with a high coloring capacity, physicochemical and organoleptic characteristics. Fresh marcs containing about 10^2 - 10^4 of microorganism cells in 1 g are suitable for making food colorants, since technological conditions used for marc processing and colorants manufacturing allow sterile end products of high quality to be obtained.

The study about the content of anthocyanin pigments in colorants made of the fresh marcs showed that chokeberry and black currant colors contain 4 anthocyanin pigments.

As seen from Figure 1, anthocyanins of the colorant obtained from chokeberry marc are presented mainly by cyanidine, cyanidine-3-glucoside and cyanidine-3,5-diglucoside. At the same time the traces of delphinidine were found in it.

In contrast to the colorant obtained from chokeberry marcs, chromatograms of the colorant from black currant marcs showed that not only spots of cyanidine pigments, but also spots of delphinidine and delphinidine-3-rutinoside were more intense (Figure 2).

Figure 1. Chromatogram of anthocyanins in the colorant of chokeberry marc:

1 - delphinidine; 2 - cyanidine; 3 - cyanidine-3-glucoside; 4 - cyanidine-3,5-diglucoside.

Figure 2. Chromatogram of anthocyanins in the colorant of black currant marc:

1 - cyanidine; 2 - delphinidine; 3 - cyanidine-3-glucoside; 4 - delphinidine-3-rutinoside.

Almost all anthocyanin pigments contained in fruits and berries were discovered in the colorants made of chokeberry and black currant marcs.

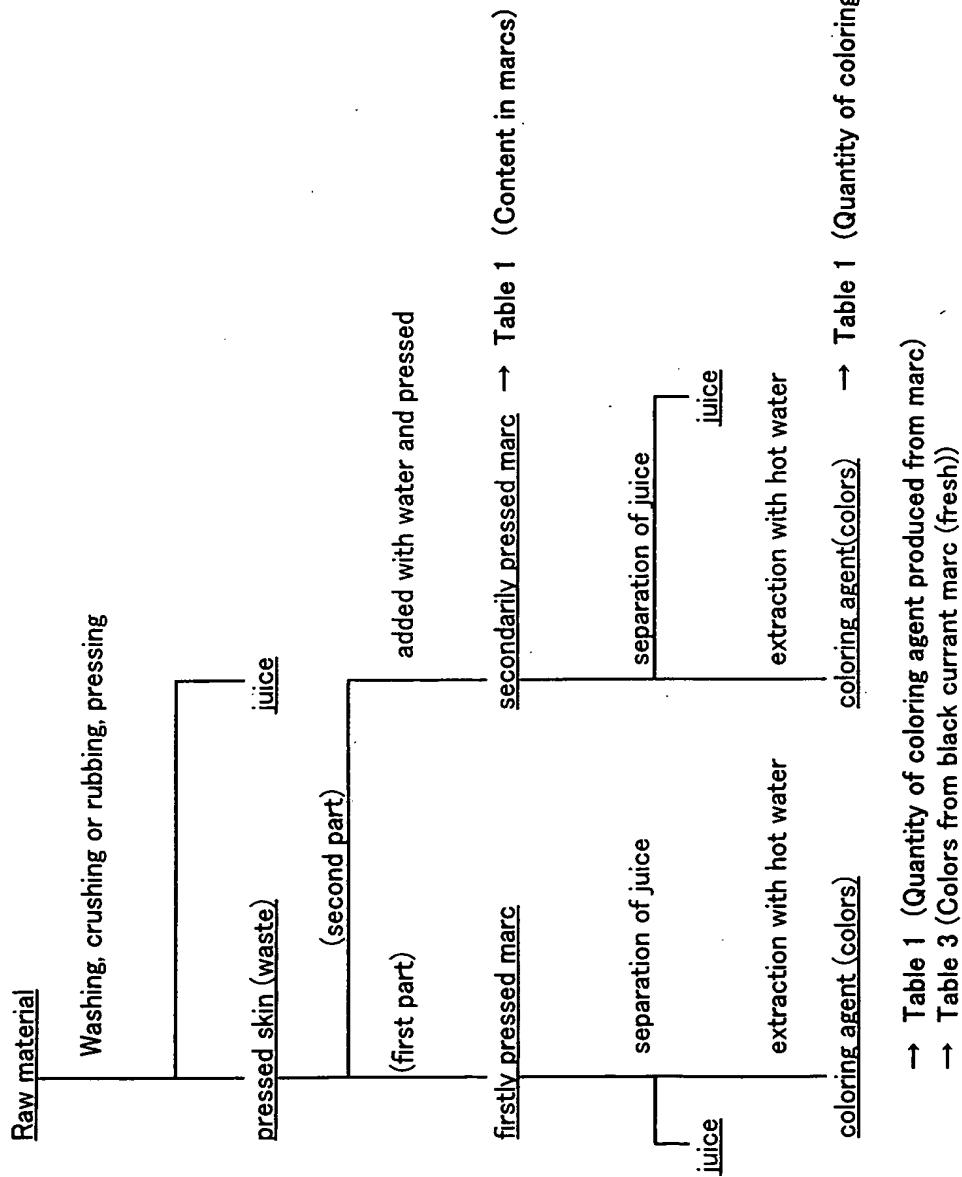
Consequently, newly pressed marcs of chokeberry and black currant are a valuable source for production of natural food colors.

Additional profit from only 200 tons of wastes used will amount to approx. 72 thousand roubles per year.

LITERATURE

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Scheme which shows the production step of the coloring agent (colors) of Nakhmedov



**U.S. Application No. 10/019,402
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Attachment 2, One Page**

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Factors influencing quantities of sugars and organic acids in blackcurrant concentrates

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Abstract Use of endogenous non-volatile flavour components, i.e. sugars and organic acids, in fruit juice products is desirable. A study of 133 blackcurrant concentrates from three seasons examined variation in sugars and acids arising from storage of fruit at freezing or sub-ambient temperature, seasonal differences, geographical origin and choice of conventional thermal-evaporative or freeze concentration technology. Compared with freeze concentrates, conventional concentrates had significantly higher contents of total sugars and acids, notably malic acid, and higher fructose/glucose, lower malic/citric acid and similar sugar/acid ratios. Concentrates from frozen fruit generally had smaller amounts of fructose, total sugars and fructose/glucose ratios than those from fresh fruit, as well as less citric, ascorbic and total acids and lower sugar/acid ratios. Principal component analysis of 40 randomly chosen concentrates showed that variance is dominated by differences in fructose, total sugars and ascorbic acid contents and sugar/acid ratios. Geographical origin and concentration technology were major sources of variance but changes in post-production sub-ambient storage could not be excluded.

Key words Fruit · HPLC · Flavour quality · Sugar/acid ratios · Principal component analysis

Introduction

Sugars and organic acids, and their ratios, play important roles in the character and quality of the flavour of fruit juice products [1]. Commercially, endogenous sugars and acids have been supplemented with sugars and starch hydrolysates, as well as organic and mineral acids [1]. However, with changes in consumer demand and labelling it has

become desirable to add only non-nutritive sweeteners to achieve acceptability.

Most blackcurrant drinks are produced from thermal-evaporative juice concentrates. During concentrate manufacture, major changes in fruit volatiles and Maillard browning reactions are favoured by increases in reactant concentrations and the elevated temperatures. Reactions between sugars and other components will continue in concentrates, albeit at reduced rates during subsequent storage at sub-ambient temperatures [2].

Alternative freeze concentration processes are now available, offering enhanced flavour quality [3]. Therefore, it is important to understand those factors that determine concentrate composition, particularly in terms of sugars and organic acids, in order to produce juice products that are perceived to have a natural character. Differences in post-harvest storage, the season of the fruit and processing technology all contribute to variation in juice products [4].

This work was aimed at establishing the significance of variation in the quantities of sugars and organic acids in thermal-evaporative blackcurrant concentrates from three seasons, prepared from either fresh or frozen fruit of various geographical origins, and comparing data with values for three freeze concentrates. This paper presents univariate and multivariate statistical analyses of data obtained by high-pressure liquid chromatography (HPLC) after sorbent extraction of the above-mentioned solutes from the concentrates.

Materials and methods

Samples. Blackcurrant concentrates ($n = 133$), processed from fruits of three seasons, were supplied by a UK manufacturer. A breakdown of samples from each season is provided in Table 1. All concentrates were stored at 4 °C in the dark.

Sorbent extraction. The method of van Horne [5] was adapted. Concentrate (1 ml) was diluted to 100 ml with deionized water, and acetic acid (AnalaR) and sucrose (AnalaR) were added, to 1 mg ml⁻¹ final concentration, as internal standards. Solutions were neutralized with a few drops of 2 M NaOH (AnalaR). Cyclohexyl (CH; 1000 mg

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Table 1 Sugar contents of blackcurrant concentrates in 1989, 1990 and 1992 seasons. (n Total number of concentrates)

Season	Post-harvest storage/ Geographical origin (n)	Fructose (mg g ⁻¹)	Glucose (mg g ⁻¹)	Total sugars (mg g ⁻¹)	Fructose/glucose ratio
1989	UK Fresh fruit (mean values) (13)	131.69	78.0	209.69	1.69
	SD	4.79	2.59	7.38	0.01
	SE	1.33	0.72	2.05	0.00
	UK Frozen fruit (32)	122.18	75.76	197.94	1.61
	SD	5.69	4.28	9.78	0.04
	SE	1.01	0.76	1.73	0.01
	New Zealand fruit (2)	169.86	112.48	282.34	1.51
	SD	1.17	0.77	1.94	0.00
	SE	0.83	0.55	1.37	0.00
1990	UK Fresh fruit (mean values) (34)	191.93	131.00	322.93	1.47
	SD	15.13	8.83	22.76	0.07
	SE	2.59	1.51	3.90	0.01
	UK Frozen fruit (10)	174.65	131.06	305.71	1.33
	SD	0.68	4.33	4.32	0.05
	SE	0.22	1.37	1.37	0.01
	Polish fruit (7)	163.20	122.40	285.60	1.33
	SD	6.76	4.34	11.10	0.01
	SE	2.55	1.64	4.19	0.00
	Imported fruit (7)	174.53	129.35	303.88	1.35
	SD	6.18	3.80	9.96	0.01
	SE	2.34	1.44	3.77	0.00
	New Zealand fruit (2)	151.11	108.43	259.54	1.39
	SD	1.57	0.45	2.01	0.01
	SE	1.11	0.32	1.42	0.01
	Freeze concentrates (3)	135.23	111.41	246.64	1.21
	SD	22.87	16.38	39.07	0.05
	SE	13.21	9.45	22.56	0.03
1992	UK Fresh fruit (mean values) (12)	169.88	137.53	307.41	1.24
	SD	25.17	13.53	29.31	0.20
	SE	7.27	3.91	8.46	0.58
	UK Frozen fruit (11)	153.56	135.54	289.10	1.13
	SD	13.35	11.43	20.40	0.10
	SE	4.03	3.45	6.15	0.03

(Varian)] and quaternary amine [SAX; 500 mg (Varian)] phase sorbent extraction columns were conditioned with column volumes of methanol and water and then mounted in tandem. Diluted concentrate (2 ml) was passed through at 2 ml min⁻¹ at 20 kPa vacuum. Filtrates were collected for sugar analysis. Top columns (CH) were then removed and SAX phases eluted with 2 ml of 0.2 M phosphoric acid (AnalaR) at 1 ml min⁻¹ at 20 kPa vacuum. Eluates were collected for analysis of organic acids.

For both groups of solutes identification was performed by preliminary HPLC analyses without internal standards, in which retention times of resolved peaks were matched with those of a number of standard pure solute solutions.

HPLC analysis. Sugars were quantified on Spherisorb NH₂ (5 × 240 mm) (Phase Separations, Clwyd, Wales) using acetonitrile/water (80/20) at ambient temperature and an ACS 740/14 evaporative mass detector (HPLC Technology, Macclesfield, England). Organic acids were determined on Spherisorb C18 (5 × 240 mm), eluting with 0.2 M phosphoric acid and monitoring effluent at 210 nm. Dry matter contents were calculated [6] and regression lines obtained using standard solutions of each identified component. Quantifications were performed in duplicate, calculating values using the internal standards.

Statistical analyses. Data were subjected to analysis of variance (ANOVA) using Minitab v8.2 and significance was inferred if *P* < 0.05. Principal component analyses were effected using Unscrambler II v3.1 (Camo A/S, Trondheim, Norway). This multivariate statistical procedure proceeds by extracting linear combinations of the original variables in data, to obtain a smaller number of variables

(principal components, or factors). The first few factors explain the maximum of variance, whilst minimizing loss of information. Relationships between samples are described in two-dimensional spaces (Figs. 1–6), in which values (scores) on the first two principal components form the *x* and *y* coordinates (product spaces), respectively. The relationships between samples and the original variables are represented by plotting the distribution of variables (loadings) on the product spaces, to yield bi-plots.

Results

Tables 1 and 2 summarize available data on blackcurrant concentrates, including: season; geographical origin, post-harvest storage of fruit and concentration technology. Quantities of sugars and organic acids, ratios between individual sugars and between organic acids as well as total sugars/acid ratios were calculated. The significance of variation due to post-harvest storage and the geographical origin of the fruit was assessed. Differences were observed both between and within 1989 and 1990 concentrates for contents of fructose and total sugars and fructose/glucose ratios but not for glucose contents. Variation within 1989 and 1990 concentrates associated with the different geographical origins of the fruit were significant for all sugar

Table 2 Non-volatile acid contents of blackcurrent concentrates in 1989, 1990 and 1992 seasons

Season	Post harvest storage/ Geographical origin	Citric acid (mg g ⁻¹)	Ascorbic acid (mg g ⁻¹)	Malic acid (mg g ⁻¹)	Total acid (mg g ⁻¹)	Citric/malic acid ratio	Sugar/acid ratio
1989	UK Fresh fruit (mean values)	156.61	8.60	9.33	174.54	16.80	1.20
	SD	9.59	0.53	0.69	10.81	0.40	0.78
	SE	2.66	0.15	0.19	3.00	0.11	0.02
	UK Frozen fruit	148.58	6.14	8.89	163.61	16.71	1.21
	SD	11.66	0.70	0.86	13.22	0.41	0.11
	SE	2.06	0.12	0.15	2.34	0.07	0.02
	New Zealand fruit	171.81	7.50	10.66	189.97	16.12	1.49
	SD	0.44	0.04	0.06	0.54	0.05	0.01
	SE	0.31	0.02	0.05	0.38	0.04	0.01
1990	UK Fresh fruit (mean values)	142.37	12.44	12.96	167.77	10.98	1.92
	SD	12.50	2.34	3.77	17.64	1.57	0.21
	SE	2.14	0.40	0.65	3.03	0.27	0.04
	UK Frozen fruit	142.36	10.46	13.30	166.12	10.70	1.84
	SD	5.23	0.99	1.30	5.73	0.86	0.06
	SE	1.65	0.31	0.41	1.81	0.27	0.02
	Polish fruit	162.24	16.75	20.87	199.87	7.77	1.43
	SD	14.94	1.41	1.69	17.12	0.46	0.11
	SE	5.65	0.53	0.64	6.47	0.17	0.04
	Imported fruit	154.06	10.92	15.32	180.30	10.06	1.69
	SD	15.77	2.51	2.70	19.92	0.85	0.22
	SE	5.96	0.95	1.02	7.53	0.32	0.08
	New Zealand fruit	148.27	9.75	14.36	172.38	10.33	1.51
	SD	1.21	7.07 e ⁻³	0.22	1.42	0.07	0.00
	SE	0.85	5.00 e ⁻³	0.16	1.00	0.06	0.00
	Freeze concentrates	128.13	3.36	0.88	132.37	145.60	1.86
	SD	24.97	2.01	0.47	26.97	53.98	0.31
	SE	0.86	1.16	0.27	15.57	31.16	0.18
1992	UK Fresh fruit (mean values)	234.36	10.08	2.47	246.91	94.88	1.25
	SD	31.26	2.35	0.76	31.10	34.34	0.18
	SE	9.02	0.68	0.22	8.98	9.91	0.05
	UK Frozen fruit	163.97	6.40	1.81	172.18	90.59	1.68
	SD	18.36	2.06	0.95	19.68	68.69	0.28
	SE	5.54	0.62	0.29	5.93	20.71	0.08

parameters. In 1989 concentrates, variations in the contents of individual (citric, ascorbic and malic) and total acids in relation to post-harvest fruit storage were significant except for citric/malic acid and sugar/acid ratios. Differences due to geographical origin were significant for all parameters except ascorbic acid content. In 1990 concentrates, post-harvest fruit storage differences were significant in terms of all organic acid parameters whereas geographical origin was correlated with significant variation only for quantities of citric and total acids and not other acid parameters.

ANOVA was performed to study the effect of concentration technology. Significant differences in the amounts of fructose, glucose and total sugars as well as all acid contents and sugar/acid ratios but not the citric/malic acid ratio were observed. Two-way ANOVA indicated that the combination of post-harvest storage of fruit and concentration technology contributed significantly to the variation in the amounts of glucose and total sugars as well as the fructose/glucose ratio but not fructose contents and all acid values except malic acid content.

For 1992 concentrates, ANOVA indicated that the amounts of fructose and citric, ascorbic and total acids varied significantly in relation to post-harvest storage as did fructose/glucose and sugar/acid ratios.

Principal component analysis was also performed on the combined sugar and acid data sets from concentrates for each season. For the 1989 concentrates, samples scores on the first two components accounted for 97% variance but showed no clear differentiation within UK concentrates on the basis of post-harvest storage of fruit (Fig. 1). There was, however, clear separation between New Zealand and UK concentrates. Loadings indicated the former group of concentrates to have high levels of fructose, glucose and total sugars. Separation on the basis of fruit storage was apparent on the third component (Fig. 2). This factor accounted for only 3% of total variance, and was highly correlated with high fructose/glucose ratios in UK concentrates obtained from frozen fruit.

Figure 3 shows a principal component analysis bi-plot for 1990 concentrates: PC1 accounts for 66% of the total variance. Discrimination was based upon differences in the amounts of fructose and total sugars as well as the fructose/glucose ratios. Among UK concentrates, those made from frozen fruit had slightly lower (but significantly different) levels of these parameters. Factor 2, accounting for 18% of the variance, did not show clear separation of samples according to their known source of variation. Loadings, however, revealed that concentrates were separated on the basis of total acids, with citric and malic acids having large

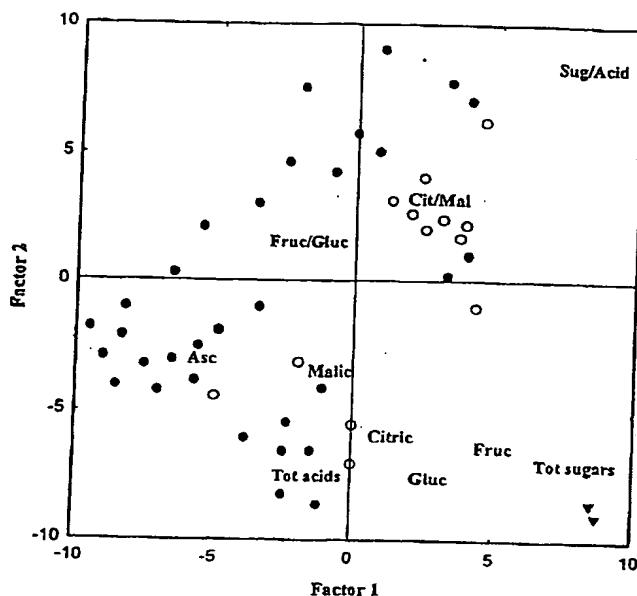


Fig. 1 Principal component analysis (PCA) bi-plot on the first and second factors, showing relationships between fresh (○), frozen (●) UK and New Zealand (▼) concentrates of the 1989 season, based on sugar and acid contents

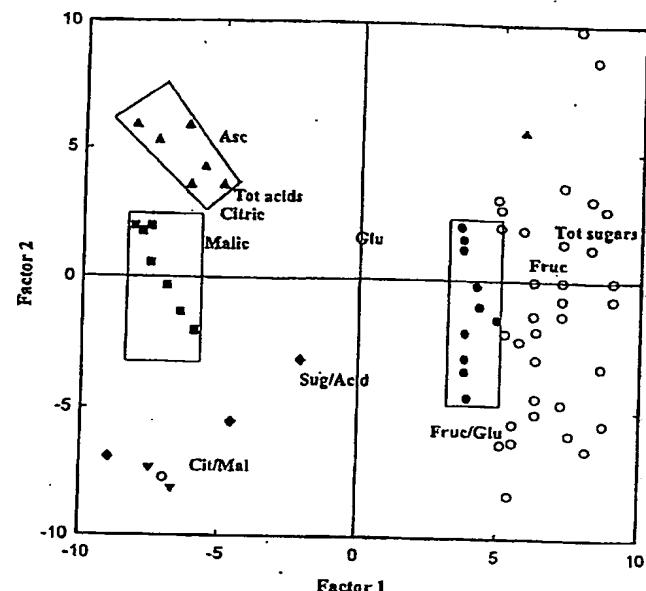


Fig. 3 PCA bi-plot on the first and second factors, showing relationships between fresh (○), frozen (●) fruit UK concentrates, imported (■), Polish (▲) and New Zealand (▼) and freeze (◆) concentrates of the 1990 season, based on sugar and acid contents

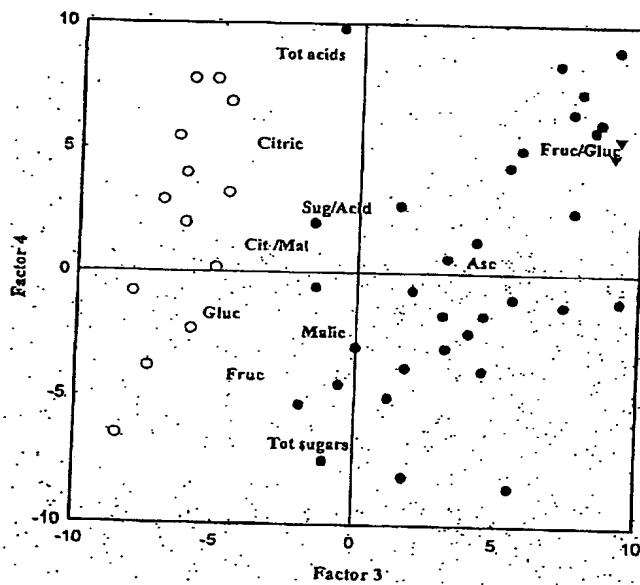


Fig. 2 PCA bi-plot on the third and fourth factors, showing relationships between fresh (○), frozen (●) UK and New Zealand (▼) concentrates of the 1989 season, based on sugar and acid contents

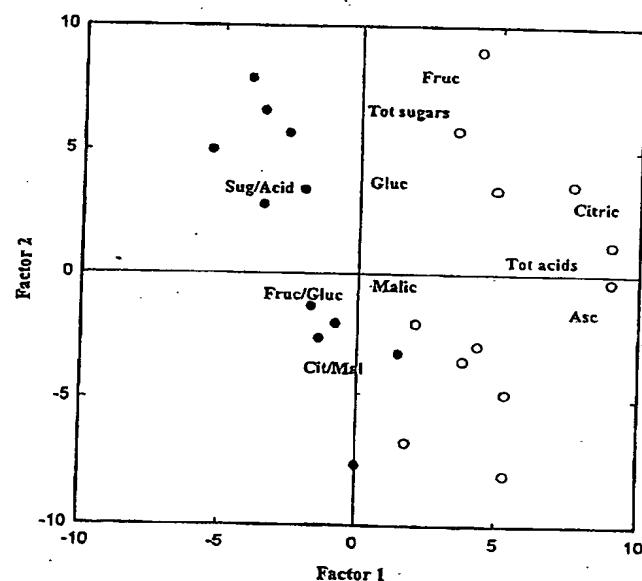


Fig. 4 PCA bi-plot on the first and second factors, showing relationships between fresh (○) and frozen (●) concentrates of the 1992 season, based on sugar and acid contents

influences. Polish concentrates were discriminated on the basis of containing relatively large amounts of total, citric and malic acids, whereas those from New Zealand contained relatively little. UK fresh-fruit concentrates were widely distributed on the basis of this component.

For 1992 concentrates (Fig. 4) PC1 explained 77% of the variance, clearly separating concentrates manufactured from fresh and frozen fruit on the basis of amount of total acids, mainly citric and ascorbic acids. In contrast, PC2 (14% of the variance), separated concentrates on the basis

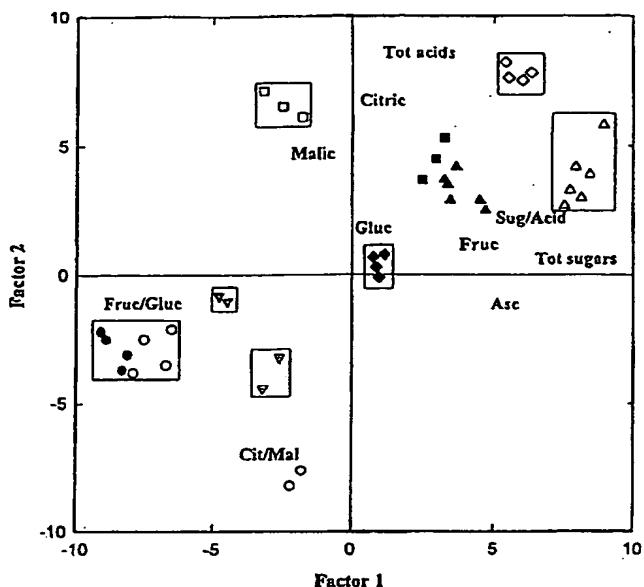


Fig. 5 PCA bi-plot on the first and second factors, showing relationships between season, post-harvest storage, geographical origin and concentration technology on sugar and acids contents of blackcurrant concentrates. (○ 1989 UK fresh fruit, ● 1989 UK frozen fruit; ▽ 1989 New Zealand, △ 1990 UK fresh fruit, ▲ 1990 UK frozen fruit, ■ 1990 imported fruit, □ 1990 Polish, ▼ 1990 New Zealand, ▭ 1990 freeze samples, ◇ 1992 UK fresh fruit, ♦ UK frozen fruit)

of their fructose and total sugar contents. This was not correlated with any known source of difference.

The results of principal component analysis on the data of 40 randomly selected concentrates from all three seasons, varying in post-harvest storage, geographical origin and concentration method, are indicated in Fig. 5. Factor 1, 37% variance, grouped UK 1990 and 1992 concentrates as well as imported concentrates and could be related to differences in fructose, total sugars and ascorbic acid contents and sugar/acid ratios. Factor 2 (19% variance) discriminated UK 1990 and 1992, Polish and imported concentrates on the basis of citric and total acid contents. Inspection of scores on PC3, 8% variance (Fig. 6), separated concentrates primarily on the basis of differences in sugar/acid ratios.

Discussion

From this study it is apparent that freeze concentrates have significantly lower contents of individual and total acids compared with conventional thermal-evaporative concentrates made from fresh berries, but similar values for citric/malic acid ratios. The effect of post-harvest storage of berries is also clear: thermal concentrates from fresh berries have significantly higher ($P < 0.050$) ascorbic acid contents ($11.1-12.4 \text{ mg g}^{-1}$) than those from frozen fruit ($9.8-10.0 \text{ mg g}^{-1}$). Fresh-fruit thermal concentrates also have significantly higher ($P < 0.050$) citric/malic acid

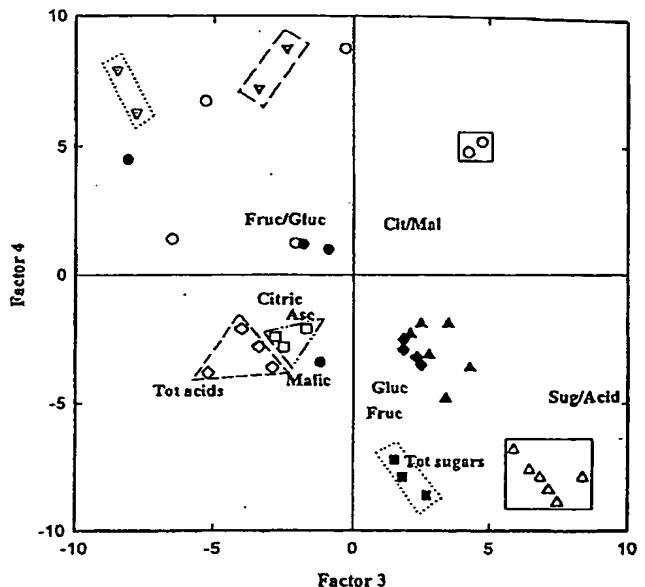


Fig. 6 PCA bi-plot on the third and fourth factors, showing relationships between season, post-harvest storage, geographical origin and concentration technology on sugar and acids contents of blackcurrant concentrates. See legend to Fig. 5 for explanation of symbols

ratios, at $11.6-12.5$, than those from frozen fruit, at $10.0-10.5$, which is a reflection of increases in malic acid content from $10.4-12.7$ to $13.5-14.5 \text{ mg g}^{-1}$. Such differences associated with post-harvest fruit storage were significant for total acids and sugar/acid ratios.

Although there were significant variations in fructose and total sugars contents associated with use of frozen fruit, glucose contents were similar. When the combination of season and post-harvest storage was considered, neither variation in fructose nor total sugars content was significant. In all three seasons the fructose/glucose ratio was significantly related with post-harvest fruit storage. When the geographical origin of the fruit was considered, only variations in the quantities of citric and total acids were significant.

Of the individual seasons, it was apparent that 1989 thermal concentrates could be discriminated into two clusters on the basis of post-harvest fruit storage, with those from fresh berries containing more fructose and, consequently, having higher fructose/glucose ratios. Concentrates from New Zealand frozen fruit contained more of all sugars, but had lower fructose/glucose ratios compared with those made from UK berries. Results for 1990 concentrates were similar; however, 1992 concentrates had lower fructose/glucose ratios compared with those from preceding years.

Variation in the sugar contents of thermal concentrates can have two origins: differences in fruit and its post-harvest storage prior to processing; and variation in the rates or extent of post-processing reactions during storage at sub-ambient temperatures. Differences in blackcurrant composition can arise from factors related to genotype,

soils and agronomic practice. During post-harvest storage of fruit, concentrate manufacture and the subsequent storage at sub-ambient temperatures, glucose and fructose will participate in the initial stages of Maillard reactions, forming Amadori and Heyns intermediates. In such reactions, glucose, the preferred substrate [7], is slowly converted to 3-substituted furans and furaneols [8–10]. Although 3-(2H)-furanones may theoretically be formed, the acidity of the concentrates does not favour such reactions [11].

Prolonged storage of concentrates, such as those of the 1989 season, was predicted to reduce ascorbic acid contents. Thomson and Fennema [12] suggested losses of up to 30% in ascorbic acid content during freezing, storage and subsequent thawing with many fruits, a view not shared by Loeffler [13] or Sulc [14]. Thomson and Fennema [12] did, however, concede that a low pH and low oxygen content may restrict losses to <5%. In the present study, 1992 concentrates had particularly high contents of all acids and relatively little variation (<3%), which could be ascribed to the use of fresh or frozen fruit. Differences between fruits are important in determining ascorbic acid losses [15], but cultivar differences could not be concluded from this study since single-variety blackcurrant concentrates were not available.

The reduced contents both of sugars and acids in freeze concentrates compared with those conventionally prepared can be ascribed to losses in ice formed during the freezing process.

Although the composition of fruit juice products may originate in differences in the original fruit, blending both of fruit, prior to processing, and concentrates is commonly done by manufacturers. The processing plant itself is a major factor that contributes to differences in fruit concentrate composition [16], but differences in post-harvest fruit storage influence respiratory activity in berries [17] and nutrient loss [18]. The absence of sucrose in concentrates may be the result of inversion, recorded to occur in blackberry [2] and apple [19, 20] concentrates. In this study glucose contents may have decreased during post-processing storage at sub-ambient temperatures, whereas the major effect in blackberry concentrates was on acid content [2]. The highest sugar/acid ratios in concentrates was observed in those made from fresh and frozen fruit in 1989; the lowest were observed to occur in 1990 concentrates, most likely a reflection of the prevailing weather at the time of harvest.

In summary, any conclusion that fresh fruit yields the best quality juice cannot be extrapolated to considerations of acids and sugars in blackcurrant concentrates [21]. Differences in the amounts of individual and total acids will influence perceptions of the flavour, character and quality of juice products. Differences in sugar contents also influence product mouthfeel, as exogenous sucrose contributes more compared with endogenous glucose or

fructose [1]. The natural non-volatiles in juice products have not been extensively studied. They may, however, play important roles in determining sensory factors such as mouthfeel and temporal characteristics of flavour [22], suggesting that more studies of the influences of differences is warranted in the search for juice products perceived to be of premium quality by consumers.

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Reduction of Acidity in Northern Region Berry Juices

Sanna Viljakainen

Dissertation for the degree of Doctor of Science in Technology to be presented with due permission of the Department of Chemical Technology for public examination and debate in Auditorium KE 2 (Komppa Auditorium) at Helsinki University of Technology (Espoo, Finland) on the 15th of May, 2003, at 12 noon.

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Abstract

Northern region berries may be used for wide variety of product alternatives as well as serving as a supply of many nutritionally valuable components. However, there are only rare, fragmentary and inconsistent scientific data available on the chemical composition of northern region berries. Therefore, comprehensive information was gathered on organic acid and soluble sugar concentrations of juices of six wild berries (bilberry, lingonberry, cranberry, cloudberry, red raspberry, black crowberry) and five cultivated berries

**U.S. Application No. 10/019,402
Appeal Brief Filed December 10, 2008**

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(black currant, white currant, red currant, gooseberry (red), strawberry) all grown in Finland. The main acids of the berry juices were invariably citric and malic acid even though their concentrations varied widely from one berry variety to another (2.9 - 16.2 g/l and 3.3 - 24.7 g/l, respectively). In addition, juices of lingonberry, cranberry, cloudberry and black crowberry contained benzoic acid (0.1 - 0.7 g/l). The main sugars of the investigated berry juices were fructose (18.0 - 57.2 g/l) and glucose (22.2 - 50.0 g/l). Most of the berries contained also sucrose (0.2 - 5.1 g/l). The data enable direct comparison of northern region berries and underline the wide variation in their organic acid and soluble sugar content, which offers possibilities for the production of numerous sensory profiles. Accordingly, the selection of the right berry for individual purposes is enhanced.

Due to their acid and sugar composition, fermentation of northern region berry juices into wines faces challenges that are not normally met when using grape juices. In berry juices the fermentations should maintain or alleviate the often rich berry aroma under conditions where the content of organic acids is high and that of fermentable sugars low. Prior to fermentation the juices have to be diluted and sugar has to be added. This causes significant weakening of the aroma and body of the wine. To reduce the acidic mouthfeel of grape wines malolactic fermentation (MLF) is widely used. However, it is not known whether MLF is applicable to modifying the acid composition of berry juices. Therefore, acid conversion by MLF by *Oenococcus oeni* was studied to improve the usability of northern region berry juices. During MLF at low pH values (pH < 4.5), malic acid was always degraded first to completion without consumption of sugars or citric acid. After the exhaustion of malic acid the degradation of both citric acid and glucose were initiated simultaneously. Thus, it is concluded that by MLF, selective conversion of malic acid to lactic acid can be achieved without loss of sugar, also in berry juices. Sequential utilization of

substrates by MLF thus enables a multitude of compositional changes in acidic juices. Control of duration of the fermentation is essential when acid reduction without loss of sugar should occur.

The most problematic compound with reference to winemaking from lingonberry is benzoic acid, which contributes to the acidity of the berry. As a microbicidal compound, benzoic acid also prevents fermentation of lingonberry juice. Thus, the known pH-dependent ability of *Saccharomyces cerevisiae* yeast to uptake benzoic acid from solutions was applied. By suspending 15 - 20 % (w/w) of the yeast for 10 min in undiluted lingonberry juice, the benzoic acid concentration was reduced by 75 - 91 %, titratable acids by about 14 % and pH increased by 0.1 units. The resulting undiluted juice was successfully fermented with a new yeast inoculum. Thus, yeast may be used as a selective absorbent to remove a certain fermentation-hindering component from the juice. These results offer new insights into berry juice fermentation.

Accordingly, MLF represents a new, promising means for acidity reduction of northern region berry juices and berry wines without a significant loss of their natural sugar content. Also, the benzoic acid uptake by the yeast was proven to be effective. By these new methods, the critical inhibitors of the further processing of the juices can be eliminated and thus it is possible to facilitate the development of various berry products of northern regions.

This thesis consists of an overview and of the following 5 publications:

1. Viljakainen, S., Visti, A. and Laakso, S., 2002. Concentrations of organic acids and soluble sugars in juices from Nordic berries. *Acta Agriculturæ Scandinavica B* 52, pages 101-109. © 2002 Taylor & Francis. By permission.
2. Viljakainen, S. and Laakso, S., 2000. The use of malolactic *Oenococcus oeni* (ATCC 39401) for

deacidification of media containing glucose, malic acid and citric acid. European Food Research and Technology 211, pages 438-442. © 2000 Springer-Verlag. By permission.

3. Viljakainen, S. and Laakso, S., 2002. Acidity reduction in northern region berry juices by the malolactic bacterium *Oenococcus oeni*. European Food Research and Technology 214, pages 412-417. © 2002 Springer-Verlag. By permission.
4. Viljakainen, S., Visti, A. and Laakso, S., 2003. Malolactic and alcoholic fermentations in black currant juice. Die Wein-Wissenschaft, in press. © 2003 by authors and © 2003 Fachverlag. By permission.
5. Visti, A., Viljakainen, S. and Laakso, S., 2003. Preparation of fermentable lingonberry juice through removal of benzoic acid by *Saccharomyces cerevisiae* yeast. Food Research International 36, in press. © 2003 Elsevier Science. By permission.

Keywords: northern region berries, organic acids, soluble sugars, acid reduction, malolactic fermentation, *Oenococcus oeni*, yeast uptake, alcoholic fermentation, berry wine, sensory quality

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